Patterns of decay caused by *Inonotus dryophilus* (Aphyllophorales: Hymenochaetaceae), a white-pocket rot fungus of oaks[1]

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Decay of living white oaks (*Quercus alba* L. and *Quercus macrocarpa* Michx.) caused by the white-pocket rot fungus *Inonotus* (*Polyporus*) *dryophilus* (Berk.) Murr. was characterized using scanning electron and light microscopy. Delignified tissues lacked middle lamellae and degradation of the cell wall was characterized by the presence of cellulosic macrofibrils. Chemical analyses showed delignified tissues to be composed of 93.47% total sugars and 2.59% lignin, whereas sound heartwood had 64.48% total sugars and 24.99% lignin. Selective delignification occurred in axial parenchyma cells surrounding vessels of earlywood and latewood. Flame-shaped tracts of vessels with accompanying axial parenchyma, present throughout the latewood, provided avenues for radial movement of *I. dryophilus*. Dense groups of latewood fibers were not degraded. *Inonotus dryophilus* did not delignify ray parenchyma or adjacent axial parenchyma; instead, a typical white rot, differentiated microscopically by a shot-hole appearance, occurred. Tyloses did not restrict *I. dryophilus* movement in heartwood vessels of living oaks. Occluded latewood fibers and medullary rays were often left intact forming borders between white pockets.

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La pourriture de chênes blancs vivants (*Quercus alba* L. et *Q. macrocarpa* Michx.), causée par la champignon de la carie blanche alvéolaire *Inonotus* (*Polyporus*) *dryophilus* (Berk.) Murr., est caractérisée en microscopie photonique et électronique à balayage. Les tissus délignifiés n'ont pas de lamelles moyennes et la dégradation de la paroi cellulaire est caractérisée par la présence de macrofibrilles cellulosiques. Des analyses chimiques montrent que les tissus délignifiés sont composés de 93,47% de sucres totaux et de 2,59% de lignine, tandis que le bois de cœur sain contient 64,48% de sucres totaux et 24,99% de lignine. Une délignification sélective se produit dans les cellules du parenchyme axial entourant les vaisseaux du bois de printemps et du bois d'été. Des groupements, en forme de flammes, de vaisseaux accompagnés de parenchyme axial se rencontrent dans tout le bois d'été et forment des voies de migration pour le mouvement radial de l'*I. dryophilus*. Des groupes denses de fibres de bois d'été ne sont pas dégradées. L'*Inonotus dryophilus* ne délignifie pas le parenchyme des rayons, ni le parenchyme axial adjacent; à la place, on rencontre une carie blanche typique, caractérisée microscopiquement par une apparence de "*shot-hole*". Les tyloses ne limitent pas le mouvement de l'*I. dryophilus* dans les vaisseaux du bois de cœur des chênes vivants. Des fibres de bois bouchées et des rayons médullaires demeurent souvent intacts et forment des démarcations entre les alvéoles blanches.

[Traduit par le journal]

Introduction

Inonotus (*Polyporus*) *dryophilus* (Berk.) Murr. causes a white-pocket rot in heartwood of living oaks. In the United States and Europe, *I. dryophilus* is a major cause of decay in several species of *Quercus* (Boyce 1961; Gilbertson 1976; Hedgecock 1912; Hepting 1971; Ryvarden 1976; von Shrenk and Spaulding 1909). Hedgecock and Long (1914) first described the mottled appearance of this decay as a whitish piped rot. They postulated that localized areas of decayed wood were composed primarily of cellulose. Few fungi can selectively remove lignin wood without destroying appreciable amounts of cellulose or other wood sugars. Microorganisms with the capacity to delignify wood have great potential for use in biological pulping process for paper production, to produce feed for livestock animals, or to release wood sugars for fermentation (Kirk *et al.* 1980).

Investigations of decay caused by *I. dryophilus* have not been done since the beginning of this century (Hedgecock and Long 1914). Recent investigation of a similar type of decay in conifers, caused by *Phellinus* (*Fomes*) *pini* (Thore ex Fr.) A. Ames, demonstrated a selective delignification of heartwood in living trees (Blanchette 1980*a*). The study reported here was undertaken to provide information on the progressive stages of delignification in deciduous tree species (*Quercus* sp.) by the heart rot fungus, *I. dryophilus*.

Materials and methods

Four bur oaks (*Quercus macrocarpa* Michx.) and one white oak (*Quercus alba* L.) approximately 50–75 years old with conks of *I. dryophilus* were cut in Anoka and Dakota counties,

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Minnesota. Trees were cut into 30-cm bolts above and below the sporophore beyond any evident discoloration or decay. These bolts were aseptically split and small sections were taken from the sporophore and all representative areas of the longitudinal surface including advanced decay, incipient decay, discolored heartwood, normal heartwood, and sapwood. These sections of wood were cultured on three different types of media; malt yeast agar (15.0 g Difco malt extract, 15.0 g Difco agar, 2 g Difco yeast extract/1000 mL H_2O), acid malt yeast agar (4 mL of 85% lactic acid added to malt yeast agar after autoclaving), and a selective medium for Basidiomycetes (Blanchette and Shaw 1978).

Radial and tangential sections of wood were cut from all representative areas of affected wood. Unfixed specimens were dried in a desiccator, and coated with 40% gold and 60% palladium in a Kinney KSE-2A-M vacuum evaporator. Specimens were observed and photographed with a Philips scanning electron microscope at 12 kV.

Blocks of wood, approximately 1.0 × 1.0 × 0.3 cm, taken from all representative areas of discolored and decayed wood, were infiltrated with water using an aspirator at low vacuum (60–65 cm mercury) for 3–5 min. After saturation, samples were embedded in a 1:1 mixture of Tissue Tek II O.C.T. embedding compound and water. Radial, tangential, and transverse sections 13–15 μm thick, were obtained using a Tissue Tek II cryostat cooled to −20°C. Sections were stained with safranin–fast green (Gram and Jorgensen 1953).

Results

Macroscopically, decayed heartwood had a white-pocket rot appearance (Fig. 1A). A cross section of an infected log (Fig. 1B) demonstrated a ring rot pattern of decay similar to *P. pini* in conifers (Shigo 1979). White pockets, observed in the longitudinal plane, were localized areas of degraded wood surrounded by apparently sound wood (Fig. 1C).

Morphological characteristics of cell wall deterioration were observed by scanning electron microscopy. A selective delignification of the wood was apparent (Fig. 1D). The absence of middle lamella between cells (Fig. 1E) and lignin removal within the cell walls (Fig. 1F) demonstrated a type of degradation different from decay by white or brown rot fungi as described by Blanchette (1980*b*). As lignin is removed, the fibrillar structure of cellulose in the cell wall becomes evident. These morphological characteristics of cell wall decay identify this degradation process as a type of selective delignification. To confirm results obtained with scanning electron microscopy, chemical analyses of white pockets from decayed wood were conducted by M. J. Effland, United States Forest Products Laboratory, Madison, WI. Total sugars and lignin contents were respectively 93.47 and 2.59% in decayed wood in comparison with 64.48 and 24.99% in sound heartwood.

The anatomy of sound oak heartwood is presented in Figs. 2A and 4. Large earlywood vessels (Fig. 2A, *v*) are surrounded by thin-walled axial parenchyma cells (Fig. 2A, *a*). Flame-shaped tracts of thin-walled cells made up of small vessels as well as axial parenchyma (Panshin and de Zeeuw 1970) occur throughout each annual ring (Fig. 2A, *t*). Thick-walled fibers are abundant in latewood (Fig. 2A, *f*). Bands of axial parenchyma occur throughout the latewood fibers (Fig. 2A, *b*). A transverse section of decayed heartwood illustrates the xylem cells most severely degraded (Fig. 2B). Large earlywood vessels (Fig. 2B, *v*) and adjacent axial parenchyma (Fig. 2B, *a*) are rapidly colonized. Delignification occurs throughout the flame-shaped tracts of cells (Fig. 2B, *t*). Thick-walled fibers (Fig. 2B, *f*) and medullary rays (Fig. 2B, *m*) remain uninfected and appear to be obstructions to decay.

During incipient stages of decay, the fungus grows vertically through earlywood vessels. Although white and bur oaks contain numerous tyloses in earlywood vessels, *I. dryophilus* hyphae colonized the vessels and penetrated the tyloses (Fig. 2C). Holes in tyloses were observed with hyphae of *I. dryophilus* passing through them (Fig. 2D). Medullary ray parenchyma cells near colonized vessels were occluded with gel- or gum-like materials (Fig. 2E). Initial formation of parenchyma occlusions was greatest in cells immediately adjacent to the vessel. Uniseriate rays also react to the fungus and undergo extensive plugging (2F). Hyphae were not found in cells with gel-like occlusions.

To determine what restricts degradation to localized areas, borders between decayed and nondecayed wood were observed. Extensively occluded medullary rays (Fig. 3A) and occluded axial parenchyma and fiber cells (Fig. 3B) separated the pockets of decayed wood. Borders between delignified cells and occluded xylary cells are indicated by arrows in Figs. 3A and 3B. A tangential section, stained with safranin and fast green, shows extensive plugging within a medullary ray (Fig. 3C). Although most ray cells are occluded, cells next to the delignified area can be degraded (Fig. 3C, arrows). In advanced decay, a degraded hourglass-shaped region of medullary ray parenchyma can be seen in cross section (Fig. 3D, arrows). In areas of advanced decay, the removal of occlusions and subsequent degradation results in coalescing of individual white pockets.

Uniseriate rays were frequently observed without occlusions. Ray parenchyma cells without plugs were readily degraded (Fig. 3E). Cell wall decay of uniseriate rays was not typically a delignification as observed in axial parenchyma cells. Instead, the cells had a shot-hole appearance (Fig. 3E, arrows) similar to white rot decay (Blanchette 1980*b*). *Inonotus dryophilus* causes two distinct types of cell wall deterioration. Axial parenchyma directly surrounding the rays were commonly

degraded in a white rot fashion while those cells further away from the rays were selectively delignified. Figure 3F demonstrates the delignification of one axial parenchyma cell and a white rot in another cell immediately adjacent to it.

To illustrate the decay process of *I. dryophilus* a three-dimensional diagrammatic representation of sound and decayed oak heartwood is presented (Figs. 4 and 5).

Discussion

Scanning electron microscopy revealed a unique micromorphological decay pattern by *I. dryophilus* in white and bur oak. Tyloses, naturally abundant in heartwood vessels, function as barriers to invading microorganisms after mechanical wounding (McGinnes *et al.* 1977; Shigo 1979). Colonization by *I. dryophilus* was not restricted by tyloses in vessels of oak. Thin-walled axial parenchyma surrounding the vessels were selectively delignified. Since incipient stages of decay appeared to be associated with the earlywood portion of an annual ring, the large size and fewer numbers of vessels and axial parenchyma do not restrict fungal movement. Colonization of the smaller and more dense latewood vessels and parenchyma was not so rapid. Vessels, smaller in size, and thin-walled axial parenchyma cells extended as flame-shaped tracts throughout the latewood (Panshin and de Zeeuw 1970). As decay progressed, *I. dryophilus* colonized the flame-shaped tracts of latewood vessels and selectively delignified the surrounding axial parenchyma. Decayed cells, constituting white pockets, were linked to adjacent annual rings by these flame-shaped tracts of cells.

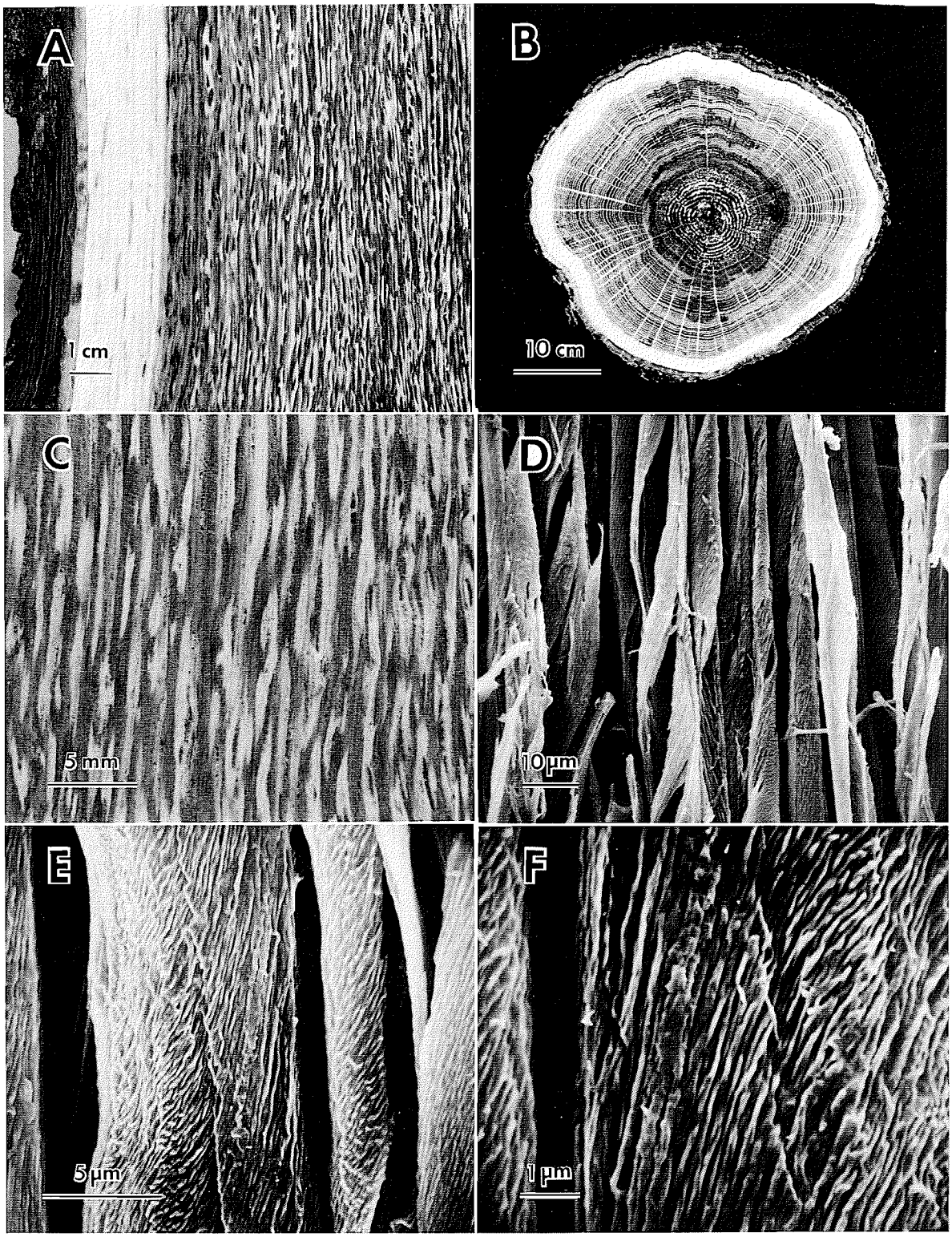
The borders of the white pockets, areas that *I. dryophilus* left undegraded, were primarily composed of medullary rays and latewood fibers. Medullary rays were found to be heavily plugged in advance of the decay and appeared to act as a morphological or chemical barrier to *I. dryophilus*. However in the most advanced stages of decay the medullary rays were degraded to an appreciable extent. As Hedgecock and Long (1914) observed, hyphae of *I. dryophilus* were capable of moving through cells alongside medullary rays. The study reported here corroborates the earlier observation of degradation in the peripheral cells of medullary rays. These outermost cells contained fewer occlusions and were more readily degraded. Colonization and degradation of these cells provides an additional avenue for *I. dryophilus* to move radially through the wood.

Thick-walled fibers which occur as a dense group of cells between the flame-shaped tracts in the latewood also act as barriers to decay. The thickness of the cell wall may create a morphological barrier to stop the fungus. Fiber cell wall thickness is attributable mainly to the S_2 layer primarily composed of cellulose (Esau 1977). The large percentage of cellulose present within fibers may make them less suitable as a substrate for *I. dryophilus*, a fungus that preferentially utilizes lignin. Investigations involving white rot fungi (Kirk *et al.* 1976; Kirk *et al.* 1978; Reid 1979) suggest that higher concentrations of carbohydrate increase lignin degradation. The increased amount of carbohydrate involved in these *in vitro* studies using white rot fungi may not trigger the same response by *I. dryophilus*. Indeed the ultrastructural study presented here and by Blanchette

FIG. 1. (A) White-pocket rot caused by *Inonotus dryophilus* in bur oak. (B) Cross section of incipient stage of *I. dryophilus* decay showing a ring rot pattern. (C) White-pocket rot with localized white degraded areas surrounded by sound wood. (D–F) Scanning electron micrographs of degraded wood from white pockets. Delignified axial parenchyma cells (D) separate owing to lack of middle lamella (E). Cell walls void of lignin contain macrofibrils of cellulosic substances (F).

FIG. 2. Cross sections of sound white oak heartwood encompassing approximately three growth rings (A) and decayed heartwood encompassing approximately six growth rings (B) demonstrating the patterns of decay within annual rings. Vessels (*v*) are surrounded by thin-walled axial parenchyma (*a*), flame-shaped tracts of cells (*t*) occur throughout the latewood and bands of parenchyma (*b*) extend through the thick-walled latewood fibers (*f*). See also Fig. 4 for diagram of sound wood. Decay begins in the earlywood vessels and surrounding axial parenchyma cells. Progressively the fungus colonizes and degrades the flame-shaped tracts of cells, uniseriate rays (*u*), and bands of parenchyma. Thick-walled fibers and medullary rays (*m*) remain unaltered. (C–F) Scanning electron micrographs of hyphae colonizing vessels and reaction of surrounding ray parenchyma cells. Hyphae in vessels of heartwood were not restricted by tyloses (C). Penetration of tyloses was evident by hyphae passing through holes in tylosis wall (D). Medullary rays adjacent to colonized vessels contained occlusions (E, enlarged area of medullary ray from Fig. 2C). Uniseriate rays were also occluded (F, arrows).

FIG. 3. Scanning electron micrographs (A, B, E, F) and light micrographs (C, D) of advanced stages of decay. (A) Occluded medullary rays (*m*) were frequently a border to areas of delignification (arrows). (B) Occluded xylary cells also acted as a barrier to *I. dryophilus* (arrows). (C) A tangential section, stained with safranin and fast green, showing an occluded medulary ray (*m*) next to delignified axial parenchyma (*a*). Degradation of medullary ray occurred immediately adjacent to delignified axial parenchyma (arrows). (D) In cross section, a medullary ray is shown that was degraded from adjacent delignified zones resulting in an hourglass-shaped region (arrows). (E) Degraded uniseriate ray cells had a shot-hole appearance similar to a typical white rot decay (arrows). (F) Axial parenchyma cells adjacent to rays also had characteristics of white rot decay (arrows) while other axial parenchyma cells, not next to rays, were delignified.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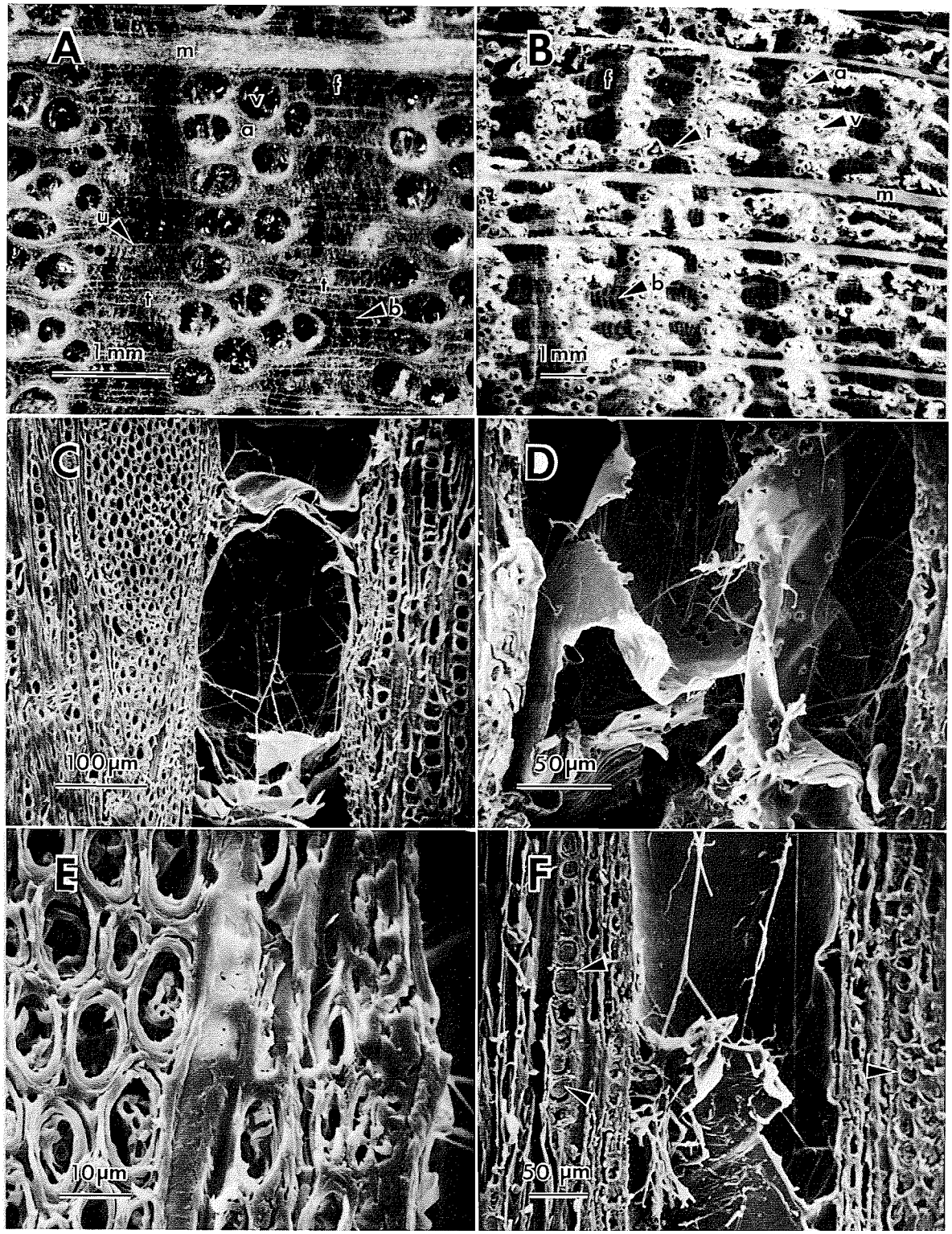
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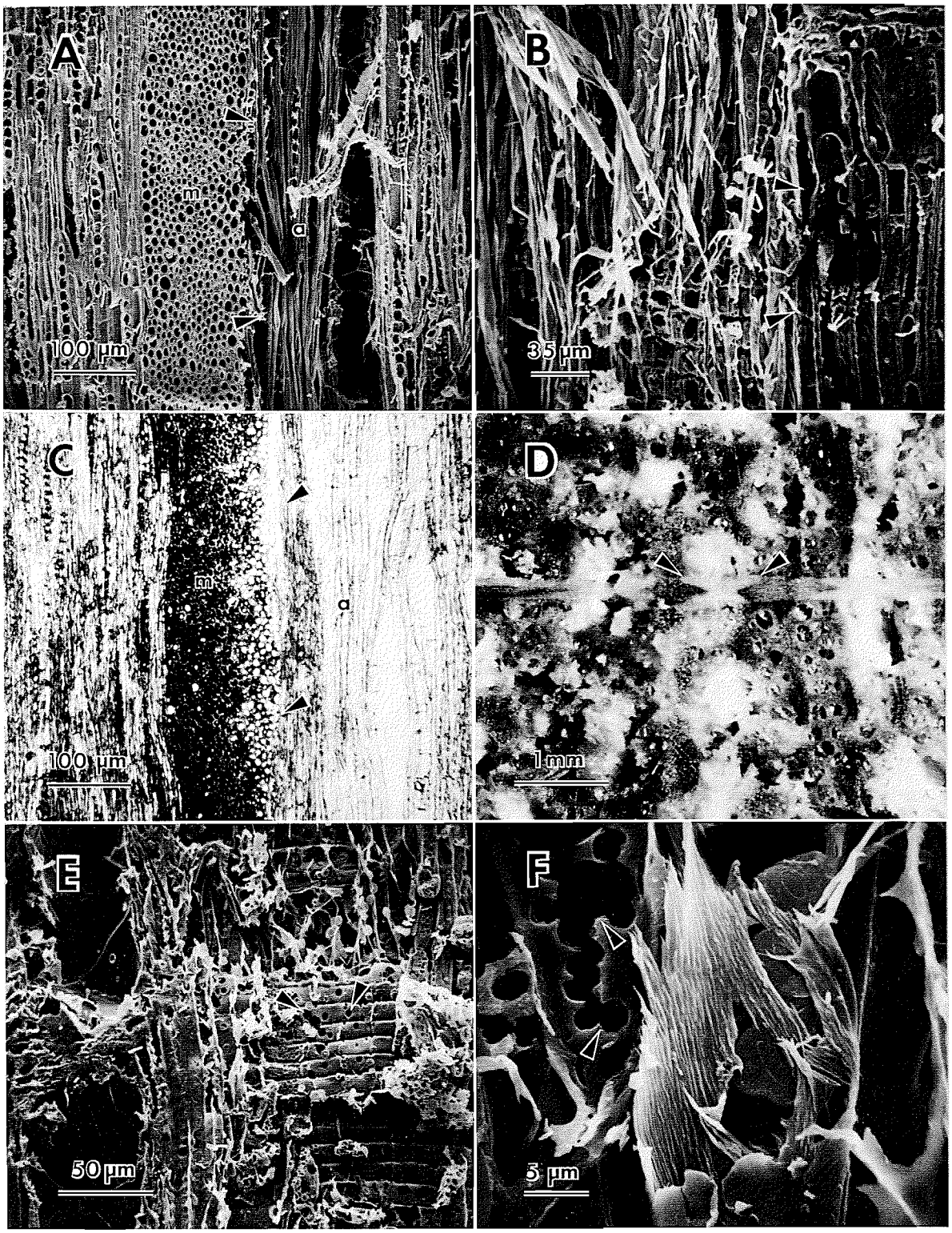
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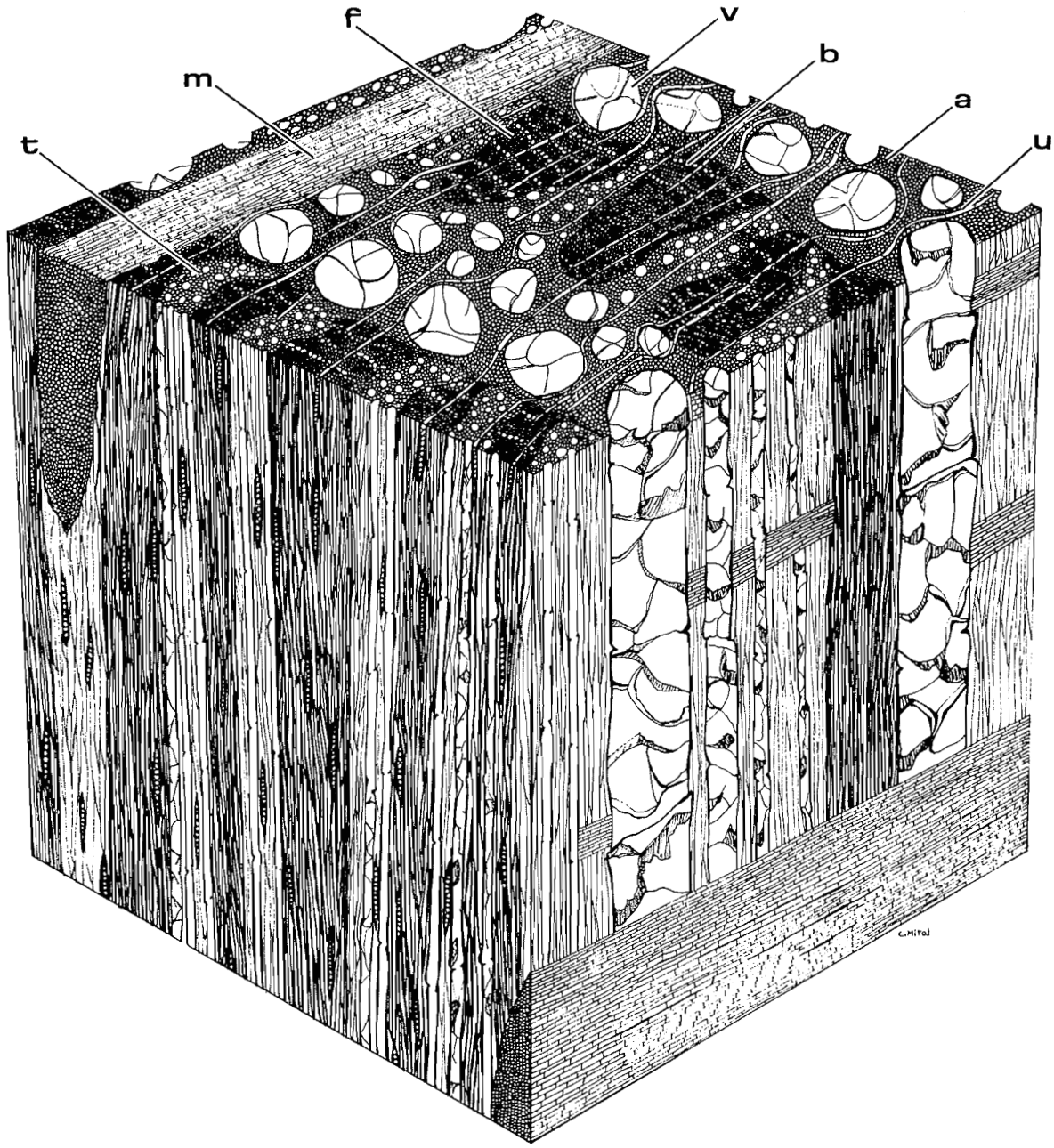

FIG. 4. A block of sound white oak (*Quercus alba* L.) heartwood. Early wood is comprised of vessels (*v*) and axial parenchyma (*a*). Flame-shaped tracts of smaller vessels and axial parenchyma (*t*) occur between dense groups of latewood fibers (*f*). Bands of axial parenchyma (*b*) perpendicular to ray parenchyma cells transect these groups of fibers. Medullary rays (*m*) and uniseriate rays (*u*) are also present.

(1980*a*) for *P. pini* in conifers demonstrated that decay by white-pocket rot fungi is different from that of white rot fungi.

One unique characteristic of white-pocket rot decay is a diffusible lignin degrading enzyme system as indicated by scanning electron microscopy (Blanchette 1980*a*). Figures 1D to 1F demonstrate the diffusible nature of the lignin degradation system by *I. dryophilus*. Delignification was observed at appreciable distances from fungal hyphae. A similar type of diffusible lignin degrading system was observed in decay of conifers by *P. pini* (Blanchette 1980*a*). Eriksson (1981) has also reported a

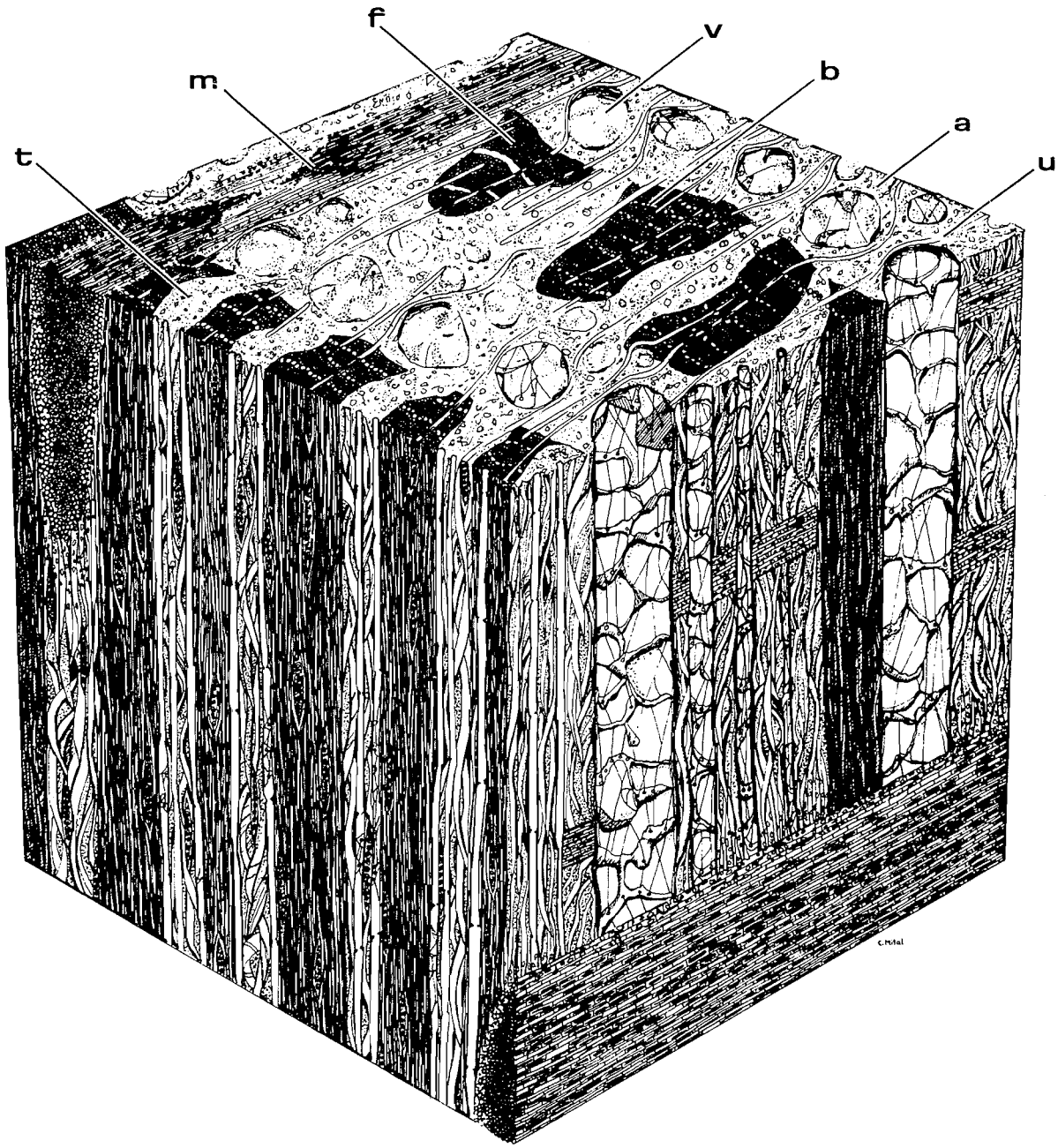

FIG. 5. A block of white oak heartwood after *Inonotus* (*Polyporus*) *dryophilus* decay. Vessels (*v*) are colonized and hyphae move easily through tyloses. Flame-shaped tracts of vessels and axial parenchyma (*t*) are degraded. Axial parenchyma cells (*a*) are delignified. Peripheral cells to medullary rays and bordering axial parenchyma cells have decay of the white rot type. Plugging occurs in the medullary rays (*m*), and forms a barrier to decay. Only in areas with concentrated amounts of hyphae are these plugs degraded sufficiently to form an hourglass-shaped pattern of decay. Uniseriate rays (*u*) are readily colonized and degraded as a white rot. Occasionally, the fungus will invade the bands of axial parenchyma (*b*) that occur throughout the otherwise sound groups of latewood fibers (*f*).

diffusible lignin modifying system and suggests that lignin modification should not be considered a contact phenomenon. Micromorphological studies have also been used to demonstrate the diffusible nature of cellulolytic degradation by brown rot fungi (Blanchette *et al.* 1978; Liese 1970; Jutte and Sachs 1976). This is in contrast to the nondiffusible lignin degradation system of most white rot fungi (Blanchette 1980*b*; Cowling 1961; Rosenberg 1980; Wilcox 1968).

Inonotus dryophilus is capable of causing two micromorphologically different types of decay. In this investigation, *I. dryophilus* degraded ray parenchyma cells and tyloses as a typical white rot and caused a selective delignification of axial parenchyma cells. The white rot caused by *I. dryophilus* was characterized by erosion troughs or holes in the cell wall associated with hyphae (Figs. 3E, 3F). This can be contrasted to delignification of axial parenchyma resulting in cellulose macrofibrils remaining after lignin removal (Figs. 1E and 1F). The loss of 90% lignin and an increase of 29% total sugars clearly demonstrates the capacity of this fungus to selectively delignify wood. However, *I. dryophilus* may degrade axial parenchyma as a typical white rot in cells adjacent to white-rotted ray parenchyma cells. This suggests that ray parenchyma affect the enzymatic capabilities of the fungus so that selective lignin removal does not occur. As the fungus degrades cells at an increasing distance from the ray parenchyma, selective lignin degradation is once again initiated. It has been demonstrated that white rot fungi degrade less lignin as nitrogen concentrations increase (Kirk *et al.* 1976; Keyser *et al.* 1978; Reid 1979). Since ray parenchyma cells are high in nitrogen compared with other cell types (Merrill and Cowling 1966), lignin degradation may be inhibited. Additional studies are needed to ascertain the ray parenchyma cell constitutents responsible for the altered capacity of enzyme activity.

In a recent study by Eriksson *et al.* (1980), bore holes were produced by a wild-type white rot fungus, *Phanerochaete chrysosporium* Burds., but not by its cellulaseless mutant. Subsequent research has shown that the enzyme systems responsible for the two types of degradation are different (Eriksson 1981). Our observations indicate that *I. dryophilus* can utilize different types of enzyme systems. Since only one type of decay is observed in a particular cell, the two different systems do not appear to occur simultaneously. Selective delignification has also been shown to proceed only with a concomitant loss of at least one type of wood sugar (Eriksson 1978). In our study, a substantial amount of cellulosic substances remain in the cell wall after decay. The loss of a particular wood sugar during the degradation process deserves further attention.

Heartwood becomes discolored in advance of *I. dryophilus* colonization. Latewood fibers from incipient stages of discolored heartwood contain few occlusions but as decay progressed the occlusions intensified. Fibers from zones of advanced decay were extensively plugged. Shigo and Shortle (1979) demonstrated that oak heartwood is capable of discoloring and reacting in respone to wounding. Wounds made into living trees were strongly compartmentalized by heartwood of healthy trees but only weakly compartmentalized by heartwood in girdled trees. In our study, oak heartwood was discolored in advance of *I. dryophilus*. As decay progressed, discoloration in wood between white pockets intensified. The accumulation of plugs in cells that border white pockets may result from polymerization products of the fungus. Hinds (1981) demonstrated that discoloration in sapwood of aspen may be the result of materials produced by fungal pathogens rather than by the host response. The accumulation of oxidized phenols can also inhibit fungal growth and decay (Highley 1975). Since these substances appear to accumulate in specific xylem cells, decay can be effectively limited to these localized areas.

Similarities were observed between *I. dryophilus* decay of white oak and *P. pini* decay of conifers (Blanchette 1980*a*). Scanning electron micrographs of delignified axial parenchyma of oak and delignified tracheids found in conifer species demonstrated similar morphological characteristics. The selective removal of lignin resulted in a fibrillar structure composed of cellulosic substances remaining in the cell wall. This type of cell wall decay appears unique to white-pocket rot fungi. Ray parenchyma cells of conifers and oaks were degraded differently from other xylary cells. In oaks, large portions of uniseriate ray cell walls were degraded by *I. drophilus*; in conifers, *P. pini* degraded the ray cells entirely (Blanchette 1980*a*). *Inonotus dryophilus* and *P. pini* were selective in the type of cells they delignified; primarily axial parenchyma in oaks and tracheids in conifers. In advanced decayed wood, heavily occluded cells separated the white pockets in both oaks and conifers. Macroscopically these fungi are responsible for a ring rot pattern of decay in cross sections of infected trees. Although these two white-pocket rot fungi occur on very different hosts the macroscopic and microscopic aspects of decay are strikingly similar.

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